MALARIAL INDEX WORK.

Methods Used in Obtaining Blood, Making Blood Smears, and Staining.

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On a recent visit to several towns and cities in Arkansas and North Carolina, where some intensive studies of malarial fevers were made, the malarial parasite index was obtained by securing specimens of blood from a number of apparently healthy individuals for this determination.

In Arkansas these were obtained from persons who were found congregated at stores, drug stores, physicians' offices, and in a house-to-house canvass. In this way specimens were obtained from 802 persons.

In North Carolina blood smears were obtained mainly from school children attending school, a few by a house-to-house canvass, and on one occasion after a public lecture. In this way specimens were obtained from 3,264 persons.

Several trials were made to obtain specimens by a house-to-house canvass, but this proved unsatisfactory and time-consuming.

The obtaining of blood smears, even after describing its purpose, is not an easy task, as the mere mention of "a drop of blood" creates fear, so that considerable persuasion and explanation have often to be resorted to, which causes much loss of time.

Public lectures and lectures to pupils assembled in the auditorium or school hall on the subject of malaria, its method of spread and prevention, and the purpose of making blood examinations to find malarial carriers explained, prompted many to submit to examination.

It was also promised that the local authorities would be informed by letter and given the names of those who were found to harbor the parasite in the blood, so that a personal, as well as a community, benefit might be derived from such an examination.

In securing blood specimens from school children, it was necessary to obtain them as rapidly as possible, so that very little interruption of class work should occur.

The method used and here described was found to be expeditious, accurate, and gave uniform results.

WORKING EQUIPMENT.

Two to three hundred clean glass slides in slide boxes.

One hundred cubic-centimeter bottle filled with alcohol.

One small package of gauze.

One Hagedorn needle stuck through a cork, with the point of the needle protruding, fitted to a small bottle of alcohol, so that the point can be quickly immersed in alcohol after each puncture.

Steel writing pen stuck in a cork.

History blanks, numbered consecutively.

The form of history blank used is as follows:

Name.....
Age.....Sex.....Color.....
Place of birth.....
Address.....
Length of residence present address.....
Previous history of malaria.....
Remarks (size of spleen if quinine taken).....
Specimen taken by.....
Date.....

Technique Employed in Making Blood Smears.

Ear lobe or end of little finger is cleaned. The use of the finger tip was found to be much more expeditious.

Puncture is quickly made with a clean Hagedorn needle.

First drop of blood is wiped off.

A drop of blood is obtained on the polished end edge of a clean slide. The edge with the drop of blood is then applied to the surface of another slide at the middle, and when the blood has spread out along the edge the top slide, held at an angle of 30° to 45°, is pushed with one stroke, fairly rapid, over the surface of the lower slide, thereby making a thin blood smear covering one-half of the slide. The film is dried rapidly and labeled immediately by writing directly into it with an ordinary soft black lead pencil the number corresponding to that on the history blank.

A smear is made over the other half of the same slide from another person in a similar manner. Thus two thin blood smears are made on one slide with an intervening clear space of one-eighth to one-fourth of an inch.

It is not necessary to label the second smear taken, as it will correspond to the next consecutive number, thereby saving time in labeling. For example, the first smear is labeled with an odd number, and the unlabeled smear on the same slide is the even or next number.

A thick blood smear is also made from each individual and two such smears spread on each slide.

The technique for making thick blood smears is as follows:

Obtain a large drop or two of blood on the surface of the glass slide which was used for spreading the thin smear, 1 inch from the end.

With the convex surface end of a steel writing pen stuck in a cork, the blood is spread evenly over a circular area about one-half to three-fourths of an inch in diameter. The pen should be immediately wiped clean after using.

A second thick blood smear is made on the surface of the other end of the same slide in a similar manner.

The first thick smear will show drying on the edge by the time the second smear is made, so that it may be labeled with a soft black

lead pencil by writing directly into it the number corresponding to the labeled (odd number) thin smear and history blank. The second thick smear will be the next even number.

The slides, thick and thin, are placed back to back, with the thick smear uppermost, and then laid in a horizontal position until dry before placing them in a slide box.

Thus is obtained one thin and one thick blood smear from each individual examined.

This method makes for a saving in time in labeling, staining, and examination, as well as a diminution by one-half of the number of slides to be handled and transported.

Two hundred and sixty-four blood smears were obtained on a single day, and often an average of 45 to 48 an hour.

Staining of Blood Smears.

Thin blood smears are fixed by immersion in pure Methyl alcohol one-half to one minute.

A Coplin jar is used, so that 10 slides, or 20 smears, may be fixed at one time.

These slides are then removed, rinsed in running water, and immersed 30 minutes or more in a stain in a Coplin jar, then air dried and examined.

The stain employed is an original Giemsa stain made up as follows, and freshly prepared:

	Cubic centimeters.
0.1 per cent watery solution of eosin	5
0.1 per cent watery solution of azur II	5
Distilled water	40

This gives a good polychrome stain, showing the parasite blue with the chromatin spots deep red. It also stains the leucocytes so that a differential count may be made.

Thick Blood Smears.

The slides with thick blood smears are immersed, 10 at a time, in a Coplin jar in a 1 per cent to a 2 per cent hydrochloric-acid solution in 95 per cent alcohol (method of James).

This solution fixes and decolorizes the films and requires from one-half to one hour.

This solution is poured off and can be reused, and the slides, after decolorization, are washed in running tap water 30 minutes, and then stained by the method as described for thin smears, then dried and examined.

The method described works automatically, requiring little attention, and the results are invariably uniform.

The label, which appears black, being carbon, is unaffected by acids, alcohol, or water, and is easily read, and, in reading the label, one knows at once on which side of the slide the smear is placed.

Examination of Slides.

All preparations are examined with a 2-millimeter oil-immersion lens, in combination with a 3x or a 4x ocular as a searcher.

Thick blood smears are examined from 5 to 10 minutes, and thin blood smears from 20 to 30 minutes.

The taking of a thin and a thick blood smear for this index work has an advantage over thick smears alone.

Thick blood films give a concentration and make for the easier finding of the presence of parasites. A good thick film is one which contains an average of 25 to 30 leucocytes to each field.

Thin blood smears require much more time for examination—about 30 minutes—but from these we can determine—

(*a*) Exact species of parasites (young forms).
(*b*) Relative number of leucocytes.
(*c*) Ascertain differential leucocyte count.
(*d*) Note any blood changes.
(*e*) Discover some other disease. (Pathological blood disease, Filaria.)
(*f*) Blood changes apparently due to malarial infection, but negative for parasites owing to effects of quinine.

The results of the findings are noted on each history blank, and, upon the completion of a series from a community, the positive findings reported by name, age, sex, and color, and type of infection found.

If in addition to the presence or absence of malarial parasites blood changes be noted, these also are made available and reported.

The benefit accruing from such examination will naturally reach the individual; as an example of this secondary diagnostic value, the finding of marked eosinophilia as possibly due to intestinal parasitic infection, so common and of such economic importance in the Southern States.